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Executive summary

Concerning re-engineering, based on deliverables from WP1 (D1.1.2.1-survey results, D1.2.4.1-results for sensory, physical and textural analysis) and WP5 (D5.1.2.1-marketing and regulatory opportunities, D 5.3.2.1 – sensory and consumer tests), a first diagnostic of product opportunities, processing problems and re-engineering options has been obtained for each product (gowé, akpan, kenkey and Kishk Sa'eedi "KS"). The main objectives and actions that will be followed for re-engineering are listed in Annexes. First results concerning re-engineering are then presented, followed by a plan of work for each product

The main conclusions for reengineering options are as follows:

(a) re-engineering will with in particular deal improving shelf life and sanitary level of the traditional products. This will go through controlling fermentation by using in particular specific functional inoculums combined with re-engineering of the other unit operations (malting, milling pre-cooking, etc) that interfere with fermentation control.

(b) Several options of re-engineering will be targeted: improvement of the "home-made" processes and traditional products, involving low capital investment processes and few modifications of the form of the original products that are targeted to the local market, and deeper process modifications and new formulations and ways of presentations of the products for larger processors and national and international markets; this includes in particular ready to prepare dried Akpan, Gowé and kenkey and new ready to drink/eat products (for all products).

Annex 1 – detailed report for Akpan

1 Diagnostic on opportunities and re-engineering option

1.1 Market opportunities and consumer demand

Based on marketing and regulatory opportunities (D 5.1.1.1 and D 5.1.2.1), 3 types of akpan will be developed: improved traditional akpan for local market, bottled akpan and akpan flour for national and international markets (Table 1).

Table 1. Akpan market opportunities

Target Consumer		Type of product	Main characteristics	Type of enterprise	Code
National level	Low income level	Traditional akpan	Improved safetiness and organolpetic characteristics Low price	Small scale traditional units[1]	Akp1
	High income level	Bottled akpan	Garantied safetiness and four weeks shelf life (4°C)	Medium[2] and large[3] scale traditional units Yogurt processors	Akp2
International level	Diaspora	Ready to prepare flour	Garantied safetiness and shelf life (12 months at ambiant temperature) Easiness of preparation	Large scale traditional units New semi-industrial units	Akp3
	Europeans				

[1] < 20 kg/month; [2] 20-60 kg/month; [3] 60-120 kg/month

The D.1.1.2.1 survey revealed two processing technologies based on submerged (Ogi) and solid state fermentation giving four types of akpan differing mainly from the raw material (maize and/or sorghum), and the way of grinding (wet milling and sieving, dry milling). The predominant technology remains that of Akpan from maize Ogi, which was used by 100% of the processors interviewed. The product presents in addition very large range variation for cooking level. The akpan should be slightly acidic, sweet/sugared, very viscous, lump free, slightly cooked (not completely) and flavoured (using citronella or vanilla extracts).

Sensory and consumer tests (D5.3.2.1) confirmed that acceptance was positively linked to milky and sweet taste, vanilla aroma and thickness, and negatively linked to fermented odour, cereal odour, and acid taste. Akpan from wet milled maize or sorghum containing sugar and/or milk were the most preferred ones.

1.2 Processing constraints

The main problems encountered during processing are safety (during steeping and fermentation, storage), reproducibility (fermentation, cooking), and product shelf life (Table 2).

Table 2. Akpan processing constraints

Code	Step				
	Intermediate product (ogi)	**Fermentation**	**Partial cooking**	**Mixing**	**Drying**
Akp1	Grain steeping/grinding Safetiness	Reproductibility Safetiness	Reproductibility Shelf life of cooked product	Cost of milk	
Akp2			Reproductibility	Cost of milk Shelf life of bottled product	
Akp3	Grain steeping/grinding and safetiness, or Grinding/sieving technology adapted to large scale units		Reproductibility		Type and cost of drying Impact on organoleptic

1.3 Physicochemical modifications during processing and physicochemical targets

Many changes occur during the process of Akpan. In particular, acidity increases while the dry matter decreases (Table 3).

Table 3: Changes in physicochemical characteristics during processing of «Akpan» from maize

Product	pH	Acidity (% lactic acid, db)	Dry matter (%, wb)	Proteins (%, db)	Ash (%, db)
Wet flour	5.83a	0.6a	46a	8.3a	1.00a
Wort	5.85a	0.7ab	34b	6.2a	1.18a
Ogui	3.97b	1.6b	28b	7.1a	0.79a
Akpan	4.09b	2.6c	14c	7.6a	1.28a
Standard deviation	1.04	0.09	14	0.9	0.21

Values in column followed by the same letter are not significantly different at 5% level

During the process characterization, some processing parameters are collected (table 4). Except for some variability, these parameters depend on Akpan types. For the Akpan made from Ogi, the usual practice for grain steeping is to pour boiled water over the grain which temperature varies between 60°C to ambient temperature (25-30°C) during the two first hours, and then is maintained at ambient temperature for 24-48h. Yield values appeared very different depending on processing technology; the lowest yield value (27%) was measured for akpan prepared from sorghum ogi. Whatever the Akpan types, the cooking duration is short, ranging from 0.1 to 0.2 hour (6-12 min).

Table 4: Processing parameters for Akpan

		Temperature (°C)	Duration (h)		Yield (% db)	
Akpan types	**Processing Steps**	**range**	**Mean or range**	**CV (%)**	**Mean**	**CV (%)**
Akpan from maize ogi *(6 producers)*	Boiling of water	92-100		-		
	Steeping {	30-60	0.5-2			
		25-30	24-48	39.4	72.8	41.8
	Fermentation	28-30	23.2	6.9	70.7	38.2
	Cooking	72-90	0.2	0.1	79.5	26.8
Akpan from sorghum ogi *(6 producers)*	Boiling of water	92-100				
	Steeping {	30-50	0.6			
		25-30	24-48	24.9	36.9	26.8
	Fermentation	28-30	23	4.9	21.9	32.5
	Cooking	95	0.1	24.4	27.4	32.8
Akpan from sorghum dough *(4 producers)*	Milling	-	0.4	17.7	97.5	1.5
	Kneading /fermentation	28-30	23.8	18.7	93	1.9
	Cooking	94	0.1	8.5	90	3.0
Akpan from maize and sorghum mixed dough *(6 producers)*	Milling	-	0.3	39.8	96.8	1.7
	Kneading /fermentation	28-30	22.9	51.3	87.4	17.0
	Cooking	90	0.2	3.1	88.5	13.1

Table 5: Organic acids and sugars identified in « Akpan » from maize ogi

Producer	Organic acid (mg/g)		Sugars (mg/g)			
	Lactic acid	Propionic acid	Maltose	Raffinose	Glucose	Fructose
Prod1	16,5	0,0	3,7	1,0	9,4	2,59
Prod2	8,7	0,0	0,77	0,58	2,1	0,73
Prod3	11,5	1,8	3,4	0,85	3,6	3,28
Mean	12,2	0,60	1,2	0,81	5,0	2,20

Akpan is a lightly cooked sour slurry, with a level (percentage) of gelatinized starch varying from 50% to 100%. Akpan is found to be slightly acidic, with pH ranging between 3.5 and 4.4. Regarding the total acidity, Akpan from Ogi (submerged fermentation) had lower acidity (whatever the raw material: 2.8-2.9% lactic acid equivalent g/100g) than Akpan from kneaded flour (solid state fermentation: 3.1% of lactic acid for mix sorghum and maize dough and 4.1 % of lactic acid for sorghum dough). Details in acid contents of Akpan from Ogi maize are summarized in table 5. Lactic acid is the main organic acid and now acetic acid is detected. In addition, during the process, many sugars are identified as a result of starch hydrolysis, but maltose and glucose are the dominant ones (Table 5).The dry matter content of Akpan varied from 15.1 and 19.6% depending on the type of Akpan. In addition to the variability due to

process type, a quite large variability is also observed between processors using the same methodology type (see table 6 for example for maize ogi type). This variability in the physicochemical characteristics can result in high inconsistency in the quality of Akpan.

Table 6: Physicochemical characteristics of «Akpan» from maize ogi

	pH	Acidity (% lactic acid, db)	Dry matter (%)	Apparent viscosity (cP)	Proteins (% bs)	Ash (%bs)
Prod 1	3,94	3,92	10,4	196	7,89	2,37
Prod2	4,18	1,9	18,3	141	7,70	0,89
Prod 3	4,37	2,16	15,3	456	7,19	1,50
Prod 4	3,86	2,8	12,6	155	7,23	1,06
Prod 5	4,31	1,6	13,7	671	7,47	1,12
Prod 6	3,89	3,1	12,1	266	7,39	0,76
Mean	4,09	2,6	13,7	314	7,48	1,28
CV(%)	5,5	33	20	67	3,6	46

Prod= producer. CV= coefficient of variation

Based on surveys and consumer tests some physico-chemical targets for the final product and during processing are indicated in table 7.

Table 7. Physico-chemical modifications during akpan processing and targets to achieve after re-engineering

Code	Step				
	Intermediate product (ogi)	**Fermentation**	**Partial cooking**	**Mixing**	**Drying**
Nat1 Nat2	Particule size (74.1< 45 µm) or 88.0<150 µm Bran level (< XX %) Free of mycotoxins Low level of pathogens	Speed of acidification Final pH (< 4.5) and Lactic acid level (7.4 mg/g) Free of acetic and butyric acids Free of pathogens	Gelatinization level (50 %) Homogeneity (no lumps)	Lactic acid level (~ 15 mg /g)	
Int1 Int2					Lactic acid level (~ 15 mg g/g) Free of pathogens

1.4 Re-engineering options

It has thus been decided to work on maize akpan, obtained from the wet process (submerged fermentation). Re-engineering will focus on (Table 8) steeping process (to shorten and secure it) and on fermentation and precooking; these steps can be indeed partly combined to improve reproducibility, safety and shelf life. Concerning fermentation, it will in particular pay attention to the kinetic of acidification that should be rapid in a first step for insuring safety but slow, in a second step to increase shelf life (consumers look indeed to products with low acidity), in particular for bottled akpan. In the case of akpan flour, it will be also tested to process it from dry dehulled and

degermed milled flour (an option that does not exist in traditional process) to decrease microbial risks linked to the wet process.

Table 8. Akpan re-engineering options

Code	Step					
	Intermediate product (Ogi)	**Pre-cooking of flour before inoculating**	**Fermentation**	**Partial cooking**	**Mixing**	**Drying**
Akp1	Maize sourcing Grain steeping for facilitating grinding	Kinetic of destruction of natural flora and preservation of starch in native state	Backsloping	Optimize (robustness)	Milk sourcing	
Akp2			Backsloping Starter culture addition	Optimize (robustness, lower acidity)	Milk sourcing Mixing before cooking (Pasteurization?)	
Akp3	Maize sourcing Grain steeping for facilitating grinding or Dry dehulling/degerming		Starter culture addition	Optimize (robustness, higher acidity)		Optimize (temperature/duration, kinetic) Choice of the type of dryer

2 Plan of work

2.1 Process upgrading

Some unit operations such as steeping process, fermentation and precooking will be upgraded.

2.1.1 Steeping process

Factors to be controlled are temperature, duration and steeping procedure. The objective of the steeping trials is to obtain a minimum steeping duration, with higher desirable particle size after milling. Based on the previous works (Ayenan,1988; Madodé et al., 2003), steeping process will be set up as followed :

- ✓ Steeping at ambient temperature (25-30°C) for 24 to 48h, with maize/water ratio of 1:2 ;
- ✓ Steeping by spilling hot water (95-100°C) over grains at a ratio maize/water of 1:2 for 6h to 24h .
- ✓ Steeping in controlled conditions (water bath at a given temperature) as described by the experimental design in Table 9

Table 9: Experimental design for precooking-steeping step

Test	Duration of steeping (h)	Temperature of steeping (°C)
1 (C)	15	38
2	9	29
3	9	47
4	21	29
5	21	47
6	6	38
7	24	38
8	15	25 (at ambient temp.)
9	15	50
10 (C)	15	38
11 (C)	15	38

Parameters to be measured: water content (kinetic of water absorption), pH, ease of grinding (particle size).

2.1.2 Fermentation

- ✓ *Kinetic of destruction of natural flora and preservation of starch in native state*

Prior to using starter culture, the kinetic of destruction of natural flora will be tested on the extracted slurry at three indicative temperatures (35, 40 and 55°C) during 8 hours.
Parameters to be measured: natural microflora counts, degree of gelatinization, acids or sugars. The temperature-duration with low natural flora counts will be selected.

- ✓ *Use of starter culture*

Selected microorganisms were tested for controling Ogi fermentation (Teniola, 2004). There are *Lactococcus rafinolactis, Lactobacillus plantarum* and *Lb.brevis*, and *Saccharomyces cerevisiae.* Firstly, these starter cultures will be bought in the commercial forms and the acidification kinetic will studied. They will be reasonably combined taking into account results obtained in the process characterization. For the kinetic study, we plan to work between 0-12h at 30°C, with sampling at 6, 8, 10 and 12h. The optimum duration for producing desirable metabolites (acids, sugars) and flora counts will be checked. Secondarily, our natural selected used flora will be used for validation. Fermentation will be set up at 30°C for optimum duration.
Parameters to be measured: pH, lactic acid, microflora count

- ✓ *Shortening/reducing the fermentation duration*

The best starter from previous test will be prepared at different ratios/concentrations starter/ogui (x, y, z et t mL/Kg) for fermenting extracted starch. The minimum or maximum level/rate of efficiency will be checked
Parameters to be measured: pH, lactic acid, microflora count

2.1.3 Cooking of sour slurry into akpan

Two options will be tested:

1- A given part/portion of sour slurry (ogi) is cooked then mixed with native slurry (second portion)
2- Partial cooking of whole slurry at intermediate temperature

In the first case (option 1), factors such as duration, temperature, ratios cooked ogi /native ogi and ogi/supernatant) will be designed. The ratios ogi /native ogi and ogi/supernatant) will vary from 2.5 to 5.3 and 2.3 to 3.4 (w/w). Temperatures of 70 and 80°C will be tested for 5 and 10 min (design A, table 10). Indeed, the gelatinization temperature of maize starch ranges between 64 and 82°C (MONNET, 2008). In addition, cooking duration observed during process characterization varied from 6 to 9 min

In the second case, the whole Ogi will be partially cooked and the ratio of ogi/supernatant, temperature and duration will be controlled. The temperature will be set up at 55 and 65°C for 3 and 5 min (design B, table 10). These two options are presented in table 1.

Tableau 10: Experimental design for cooking

Test A	Temperature (°C)	Duration (min)	ogi 1/ogi2	Supernatant/ogi 1 (v/v)
1	70	5	5	2.8
2	70	10	5	2.8
3	80	5	5	2.8
4	80	10	5	2.8
5	70	5	6	2.2
6	70	10	6	2.2
7	80	5	6	2.2
8	80	10	6	2.2
9	70	5	4	3.7
10	70	10	4	3.7
11	80	5	4	3.7
12	80	10	4	3.7
13	70	5	3	2
14	70	10	3	2
15	80	5	3	2
16	80	10	3	2

Test B	Temperature (°C)	Duration (min)	Supernetant /Ogi (v/v)
1	55	3	3
2	55	5	3
3	65	3	3
4	65	5	3
5	55	3	4
6	55	5	4
7	65	3	4
8	65	5	4

Parameters to be measured: degree of gelatinization, pH, lactic acid, microflora count, apparent viscosity

2.2 Presentation forms of Akpan

Two new forms of presentation will be tested: bottled yoghurt like akpan form and dried ogi.

2.2.1 Bottled yoghurt like akpan

Akpan obtained in the optimum condition will be diluted together with the other constituants (table 11) and bottled in 33 cL plastic containers as for yoghurt. The storage behaviour during 5 to 20 days will be tested through physicochemical parameters (pH, acid lactic) and microbiological counts. In addition, acceptability test will be performed on samples with minimum risks of safety.

Tableau 11: Experimental design for bottled Akpan

Test	Akpan Nature (g)	Sugar (g)	Milk (g)	Water (g)
1 (témoin)	60	10	7	23
2	56.8	5.7	8.6	28.9
3	57.5	9.94	8.6	30.6
4	57.3	9.91	9.1	30.5
5	57.7	9.97	7	30.6

Parameters to be measured**:** dry matter content, pH, total acidity, viscosity, microflora counts, acceptability

2.2.2 Dried Ogi

Wet Ogi will be dried at 45°C or 65°C to obtain flour containing about 10% water (Table 12). The dried Ogi will be milled and tested for reconstitution into Akpan.

Table 12: Experimental design for drying Ogi

Test	Duration (h)	Temperature (°C)
1	12	55
2	4	55
3	10	65
4	6	45
5	10	45
6	6	65
7 (C)	8	55
8 (C)	8	55
9 (C)	8	55

Parameters to be measured**:** dry matter content, pH, total acidity, viscosity, microflora counts, acceptability, Particle size, colour

Annex 2 – detailed report for Gowé

1 Diagnostic on opportunities and re-engineering option

1.1. Market opportunities and consumer demand

Based on marketing and regulatory opportunities (D 5.1.1.1 and D 5.1.2.1), 3 types of gowé will be developed: improved traditional gowé for local market, bottled gowé and gowé flour for national and international markets (Table 1).

Table 1. Gowé market opportunities

Target Consumer		Type of product	Main characteristics	Type of enterprise	Code
National level	Low income level	Traditional gowé with improved shelf life	Improved safetiness and organolpetic characteristics 2-4 weeks shelf life Low price	Small scale traditional units[1]	Gow1
	High income level	Ready to drink bottled gowé	Garantied safetiness and 4 weeks shelf life (4°C)	Medium[2] and large[3] scale traditional units	Gow2
International level	Diaspora		Garantied safetiness and shelf life (12 months at ambiant temperature)	Large scale traditional units New semi-industrial units	Gow3
	Europeans	Ready to prepare flour			

[1] < 45 kg/week; [2] 45-70 kg/week; [3] 70-100 kg/week

The D.1.1.2.1 survey revealed eight types of processes to obtain gowé that differ mainly from the raw material (sorghum red or white, and/or maize), malting conditions and use (level), fermentation condition (one or two steps, duration) and cooking conditions (pasting or steam cooking). The main criteria of acceptance for gowé are red colour, sweet and moderate acidic taste, cereal aroma and thick and smooth texture. It must also be free from moulds that appear during storage when wrapped in vegetable leaves without proper heat-treatment.

Sensory and consumer tests (D5.3.2.1) confirmed that sweetness is the first indication of acceptability for gowé and that gowé with sugar from malted and non malted sorghum with two fermentation steps was the preferred one by African consumers, followed by gowé with sugar from malted sorghum and non malted maize. European consumers will accept two fermentation steps gowé added with sugar and milk or with sugar alone. This allows masking the fermented aroma and acidic taste of the product.

1.2. Processing parameters and processing constraints

The main problems encountered during processing (D5.1.2.1) are safety (during malting and fermentation), reproducibility (fermentation, cooking), duration (malting) and product shelf life (Table 2).

Table 2. Gowé processing constraints

Code	Step					
	Germination	Saccharification	Partial Cooking	Fermentation	Final cooking	Drying
Gow1	Reproductibility Safety Duration	Reproductibility Safety	Reproductibility	Fermentation control Safety Shelf life of fermented product	Shelf life of cooked gowé	
Gow2						
Gow3				Fermentation control Safety		Type and cost of drying Impact on organoleptic quality Safety of dried product

As for Akpan beverage, high variability was observed during Gowe processing, mainly during soaking, malting and drying steps. Indeed, the soaking duration varied between 10 to 17h, with water absorption of grains varying from 17.2 to 34.7% (wet basis). The malting and drying duration ranging from 24 to 48h and 3 to 12h respectively. Consequently, this high variability of malting duration affects the functional properties of the malt. The grain can also be contaminated by moulds.

Originally, malted grains and non malted grains were mixed for Gowe production. Recent data collected during process characterization showed a large range of 20 to 39% of malted grains against 61 to 80% of non malted grains.

1.3. Physicochemical modifications during processing and physicochemical targets

Tables 3 summarizes processing parameters for Gowe obtained through malting, saccharification and fermentation steps while Table 4 and 5 present changes in some physicochemical parameters during the processing of Gowe.

Table 3. Selected processing parameters during Gowe production

		Temperature (°C)	Duration (h)	Yield (% db)
Gowe types	**Processing Steps**	**range**	**range**	**Mean**
Gowe sorghum *(fermentation +saccharification)*	Soaking	28-30	10-15	93
	Germination	28-30	24-48	79.1
	Sundrying	30-33	3-6	76.7
	Milling	-	-	72.1
	Kneading (1 :1 w/w)	28-30	5-10	-
	Saccharification	28-30	4-6	64.2
	Fermentation	28-30	12-24	56.4
	Cooking	75-85	20-45	44.0
	Cooking	90	0.2	88.5

Table 4: Changes in physicochemical characteristics during Gowe production

Types of Gowe	Products	Dry matter (% wb)	pH	TTA (% lactic acid)	Viscosity (uRVA)	Tannins (% db)	Ash (% db)	Protein (% db)
Sorghum	Sorghum	87.1 ± 0.3				0.07± 0.01		10.7 ± 0.3
Gowe	Malted sorghum	85.3 ± 1.1				0.05± 0.01		11.7 ± 0.5
(N =6)	Malted/non malted sorghum	86.5 ± 0.6	6.4 ± 0.2	0.7 ± 0.1		Nd	1.8 ± 0.4	10.7 ± 0.4
	Raw Gowe	28.9 ± 1.9	4.0 ± 0.2	5.6 ± 1.8		0.05 ± 0.01	1.4 ± 0.5	13.1± 0.5
	Cooked Gowe	23.5 ± 5.0	4.4 ± 0.1	5.3 ± 1.4	252 ± 125	0.05 ± 0.01	1.4 ± 0.6	12.5± 0.2
Maize	Maize	88.8 ± 1.0	Nd	Nd		0.05± 0.01		7.5 ± 0.3
steam	Malted maize	85.9 ± 0.8	Nd	Nd		0.04± 0.009		7.8 ± 0.2
Cooked	Malted / non malted maize	87.1 ± 0.9	6.3 ± 0.3	0.9 ± 0.1		Nd		Nd
Gowe	Raw Gowe	35.3 ± 8.9	4.6 ± 0.2	3.5 ± 0.8		0.04± 0.01	1.0± 0.3	9.1 ± 0.5
(N =4)	Cooked Gowe	35.6 ± 1.2	4.6 ± 0.2	2.5 ± 0.2		Nd	1.1± 0.2	Nd
	Steam cooked Gowe	35.0 ± 1.7	4.5 ± 0.1	2.2 ± 0.2	201 ± 88	0.04± 0.008	1.0± 0.2	9.3 ± 0.3

Table 5: Sugar and organic acid (mg/g db) identified during production of sorghum Gowe

Sorghum Gowe	Citic acid	Lactic acid	Acetic acid	Raffinose	Sucrose	Glucose	Fructose
Malted/non-malted flour	3.0±2.0	-	-	1.7 ± 0.1	9.2 ± 6.0	6.4 ± 1.4	3.6 ± 0.9
Raw Gowe	-	25.9 ± 10.0	-	3.7 ± 2.6	4.2 ± 8.1	27.8 ± 33.0	3.4 ± 3.4
Cooked Gowe	-	36.2 ± 8.9	1.9 ± 3.8	7.4 ± 4.3	12.9 ± 13.6	43.3 ± 31.3	9.5 ± 13.6

Gowe is sugary and sour beverage with pH ranging from 4.0 to 4.2 for sorghum Gowe and from 4.4 to 4.7 for maize Gowe. Regarding the titratable acidity, sorghum Gowe had higher acidity (5.3% as lactic acid) than maize Gowe (2.4% as lactic acid). The dry matter varied from 19.2 to 28.7% for sorghum Gowe and 17.8 to 25.5% for maize Gowe. The apparent instant viscosity ranged between 160 to 463 cP for sorghum Gowe and from 134 to 423 cp for maize Gowe.

Table 6 tentatively lists the main features looked during Gowé processing and physico-chemical modifications during gowé processing and targets to achieve after re-engineering

Code	Step					
	Germination	Saccharification	Partial Cooking	Fermentation	Final cooking	Drying
Nat1	Grain colour (red) Particule size (40%< 45 µm) Free of mycotoxins Low level of pathogens	Speed of acidification Final pH (λ4.5) and Lactic acid level (λ10 mg/g db) Free of acetic and butyric acids	Gelatinization level (XX %) Homogeneity (no lumps)	Sugar level (6100 mg/g) Lactic acid level (630 mg/g db) Low level of pathogens	Starch hydrolysis level Sugar level (70-100 mg/g) Lactic acid level (6 20 mg/g)	
Nat2						
Int1						Starch hydrolysis level Sugar level (70-100 mg/g) Lactic acid level (6 20 mg/g)
Int2						

1.4. Re-engineering options

It has thus been decided to work on gowé prepared from pure red sorghum including malting. Re-engineering will focus on (Table 7) malting process (to shorten and secure it) and on the couple saccharification/fermentation and cooking; these steps can be indeed partly combined and/or their order reversed to improve reproducibility, safety and shelf life. Concerning fermentation, it will in particular pay attention to the relative kinetics of acidification and alpha-amylase activity. It has indeed be proved that acidification rate during fermentation plays a crucial role on the sweetness of the product as low pH inhibits amylase activity (see first results). The kinetic of acidification during shelf life is also a crucial point that will be addressed in the work-program of re-engineering. The growing and acidification property of various LaB strains was recently modelled and this will be helpful for the choice of the proper LaB strains. In addition, i) in order to improve nutritional and sensorial qualities of gowé, a dehulling step will be tested, and ii) vacuum packaging of gowé will be tested in order to improve shelf life of the traditional form of gowé. Steam cooking can also be combined with vacuum packing to increase shelf-life of the traditional form of gowé.

Table 7. Gowé re-engineering options

Code	Step						
	Germination	Mixing of malted and no-malted grains	Saccharification	Partial cooking	Fermentation	Final cooking	Drying
Gow1	Grain sourcing (high amylase potential) Optimize grain soaking Optimize germination (duration, drying) Test grinders (hammer mill etc)	Dehulling of no malted grains Optimize mixing (ratio malted grains/dehulled non malted grains)	Optimize (temperature, duration, inoculation with LaB)	Optimize (temperature, duration, dry matter and water levels)	Optimize (inoculation, duration)	Optimize cooking and wrapping with leaves (steaming, vaccuum packaging?)	
Gow2						Optimize cooking	
Gow3					Optimize (inoculation, duration, temperature)		Test available dryers

2 First results

We first tried to understand physico-chemical modifications occurring during the main operation units in the perspective of their optimisation.

2.1. Malting

Optimum malting conditions have been defined for sorghum grains at laboratory level. Grain is first soaked for ~15 hours at 30°C to get moisture content between 45-50% (wb). Germination is then achieved at 30°C with high moisture conditions (relative humidity over 95%) for 72 hours. Drying can be performed at relatively high temperature (for example 60°C) to rapidly lower moisture content for hindering mould development and amylase degradation. Germs can then be eliminated by friction and sieving; they are indeed very rich (more than 100 mg/kg) in cyanids. In these conditions, alpha amylase activity can be of ~ 100 units/g (db, Ceralpha units) and beta-amylase activity of ~ 3 units/g (Betamyl units). Dry matter losses are of 12-16% (db); ~ 2% during soaking, 5% for germination and 6% for sprout elimination.

Water content during germination is a major parameter; alpha-amylase development is positively correlated with water content and seems to be optimum for ~ 55% (Figure 1). But the higher the moisture content, the higher the risk for mold development.

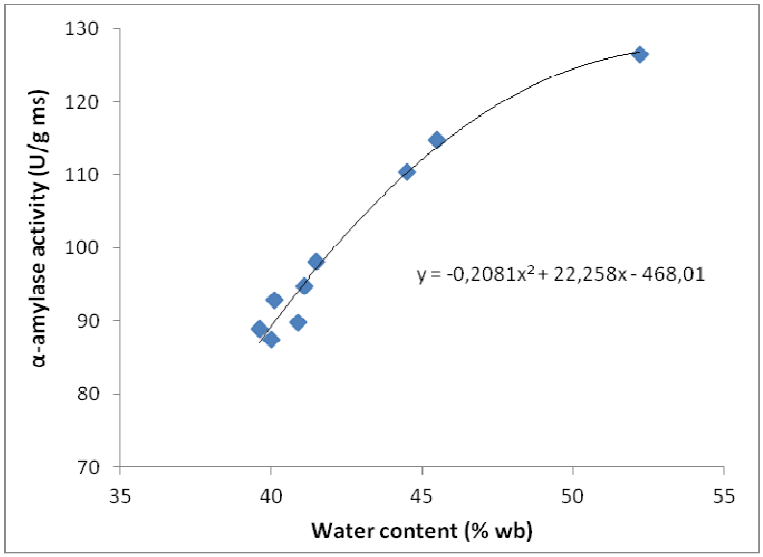


Figure 1. Relationship between water content after soaking and amylase activity after full germination for 72 h

Several attemps for limiting this risk have been tested such as addition of salt during soaking, or use of Lactic acid bacteria during soaking. But this must be more deeply studied in the following of the work.

2.2. Fermentation(s) and pre-cooking

Fermentation is a key operation unit; acidification must be rapid and intense to obtain a safe product with high shelf life, but limited to lower acid taste and fermented aroma that are rejected by some consumers. One or two fermentation steps (the first one being called saccharification step) are traditionally used without any inoculation. In addition, one part of the product is cooked before fermentation.

We thus compared gowé produced without or with inoculation with *Lactobacillus plantarum* strain, using the one or two step fermentation process.

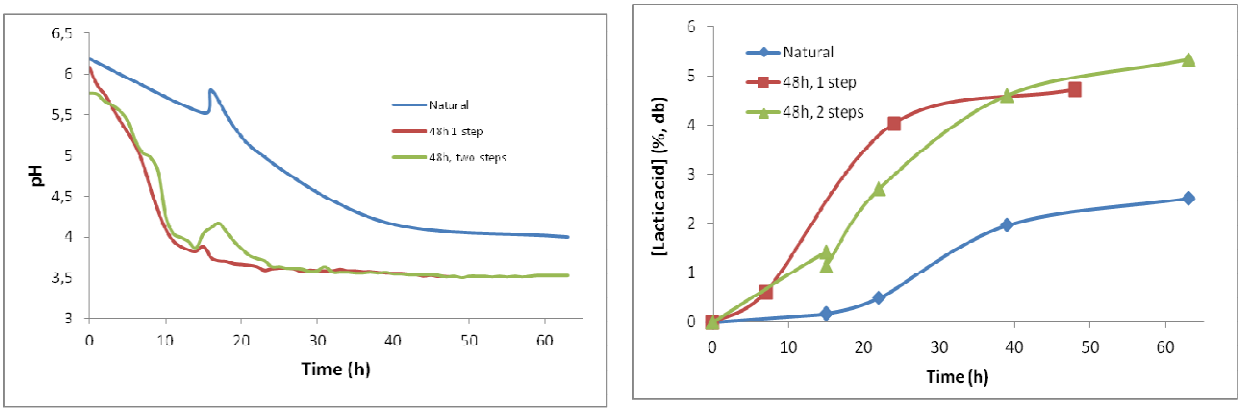


Figure 2. Evolution of pH and lactic acid content during natural or *Lb plantarum* inoculated 48 fermentations, with 1 or 2 step process

Inoculation (at 10^6 CFU/g) dramatically speeds up and promotes acidification (Figure 2). In a saftey perspective, fermentation can be stopped (pH under 4.5) after ~ 10 h when inoculation was used and after ~ 30 h when natural fermentation was applied.

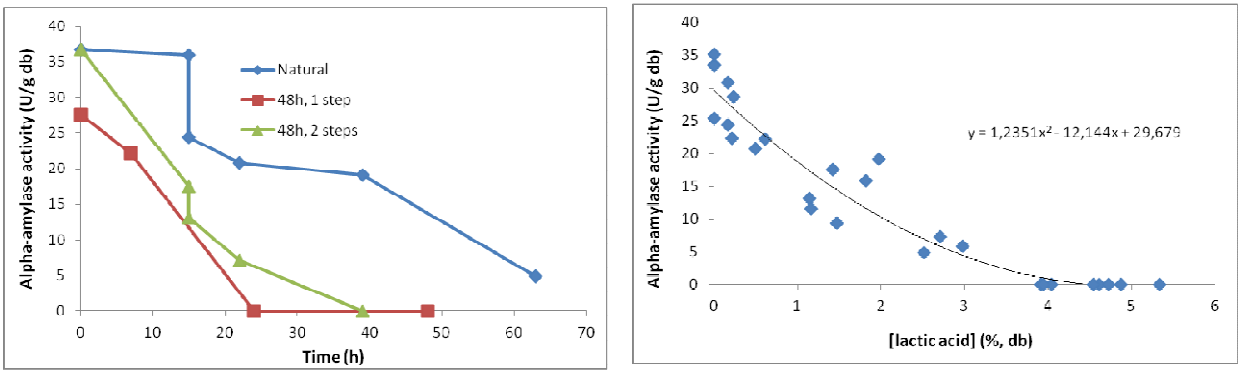


Figure 3. Evolution of alpha-amylase activity during fermentation and with lactic acid content.

Alpha-amylase activity rapidly decreases for inoculated fermentations and was null after 24-30 h (Figure 3); the decrease is much slower in the case of natural fermentation and a residual activity remains at the end of the fermentation. Alpha-amylase activity appears inhibited when lactic acid content was over 4 % (db), ie for a pH lower than 4. Cereal alpha-amylases are indeed known to be irreversibly degraded in acidic conditions.

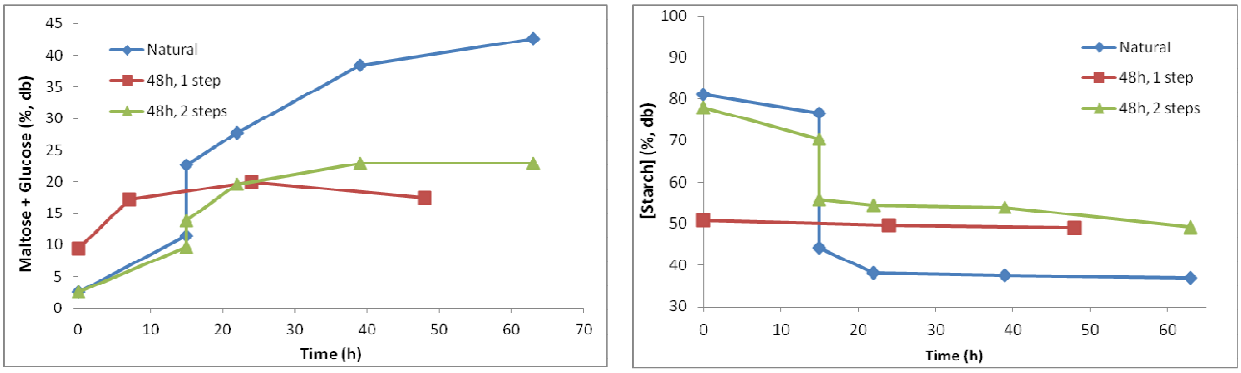


Figure 4. Evolution of maltose + glucose and starch contents during fermentation.

Maltose and glucose concentration rapidly increases for natural fermentation (Figure 4) to reach 40-45% (db). In the case of inoculated fermentations, maltose and glucose concentration remains lower than 25%. In parallel, starch content decreases to less than 40% for natural fermentation but remains close to 50% for inoculated fermentations. These differences appeared clearly linked to the inhibition and degradation of cereal alpha-amylase previously synthesized during malting by the acidic pH generated during fermentation by the inoculum.

As a whole, inoculated gowé will present higher acid taste and lower sugary taste; attributes that will not be accepted by consumers. It is thus a great challenge to develop a process combining safety and reproducibility (implying inoculation and rapid acidification) and good sensorial attributes (that suppose keeping alpha-amylase active as long as possible).

We thus tried to modelize the fermentation process in order to simulate various fermentation and inoculation conditions and their impact on amylase activity, ie on expected organoleptic quality; the higher will be the residual amylase activity, the higher will be the sweetness.

2.3. Modeling fermentation & impact on alpha-amylase activity

Gowé processing can be oversimplified as follows (Figure 5). Enzymes, particularly alpha- and beta-amylases, are produced during malting. They hydrolyze starch, thus lowering gowé paste viscosity after cooking, and giving fermentable sugars (mainly maltose and glucose). These sugars are used by Lactic acid bacteria for growing (reaction 1) and production of lactic acid (reaction 2). This will cause pH decrease (reaction 3) that will lower Lb growing (reaction 4) and amylase activity (reaction 5). We will first focus on the modeling of the 5 reactions outlined above. This has been done for 3 industrial Lb strains: *Lb plantarum*, *Lb casei* and *Lb brevis*.

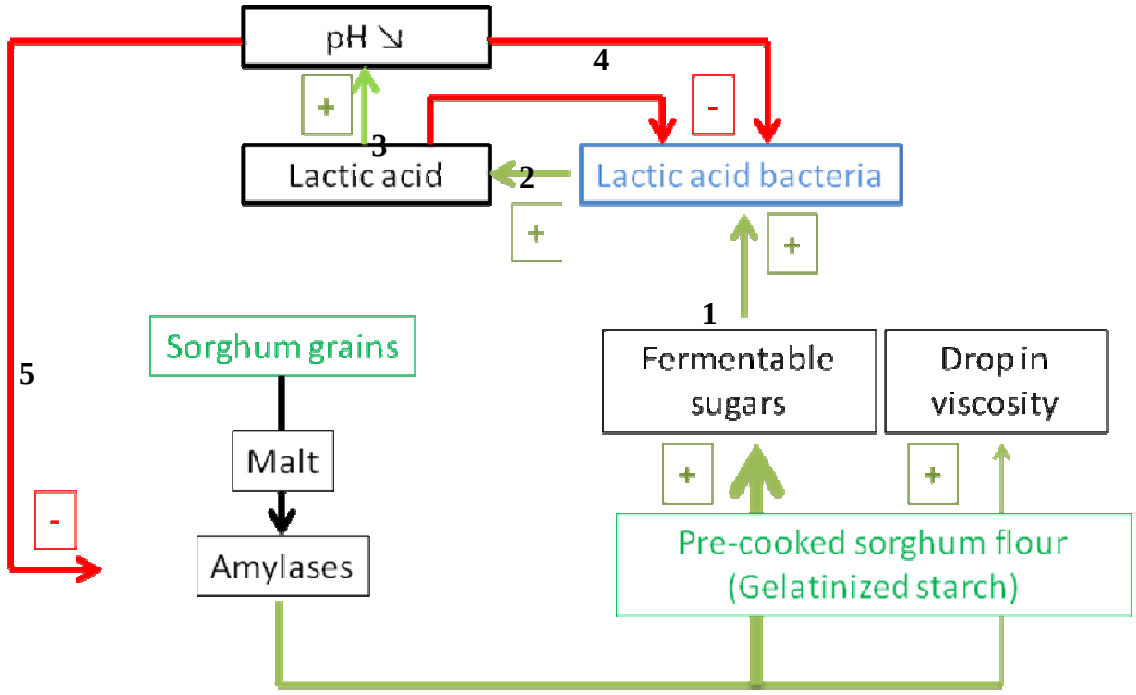


Figure 5. Outline of main reactions in gowé processing

We used a two steps cardinal approach to modelise bacterial growth. In a first step, a primary logistic model with lag time (Rosso et al., 1996) was used:

$$\begin{cases} \frac{dx}{dt} = 0 \; si \; t \leq lag \\ \frac{dx}{dt} = \mu max \times \left(1 - \left(\frac{x}{xmax}\right)\right) \times x \quad si \; t > lag \end{cases}$$

Where μmax is maximum growing rate, *lag* is lag time, *x* and *xmax* are population density (CFU/g) at time t and maximum, respectively.
The parameters have been adjusted to the experimental growing curves of each strain (reaction 1) cultivated on MRS medium buffered at various pH conditions (Figure 6).

In a second step, the influence of environmental conditions on growing rate is expressed by a cardinal model:

$$\mu max = \mu opt * \gamma(pH) * \gamma(malt) * \gamma(temperature)$$

Where μopt is the maximum growing rate for MRS medium at optimal pH and $\gamma(pH)$, $\gamma(malt)$, and $\gamma(temperature)$ the interaction contribution of pH, malt medium and temperature. Any other factor can be added in the model with the same strategy. We will first only consider interaction of pH and substrate (malt). A secondary cardinal pH model (CPM) has been applied to fit with the variation of µmax for each pH (reaction 4; Rosso et al., 1995; Figures 6 & 7):

$$\gamma(pH) = \begin{cases} pH < pH\ min, & 0 \\ pH\ min < pH < pH\ max, & \dfrac{(pH - pHmin)(pH - pHmax)}{(pH - pHmin)(pH - pHmax) - (pH - pHopt)^2} \\ pH > pHmax, & 0 \end{cases}$$

Where pH_{min}, pH_{max} and pH_{opt} are minimal, maximal, and optimal pH for growing.

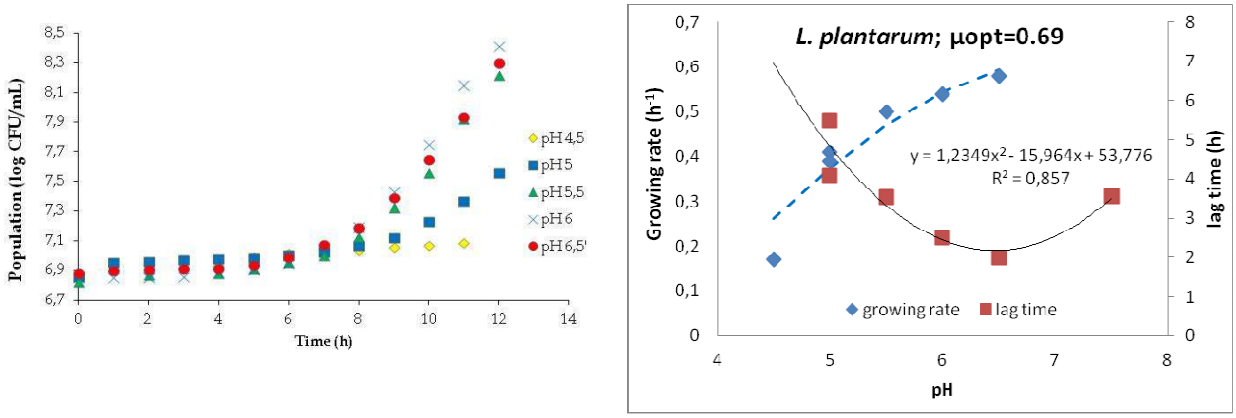


Figure 6. Growing curve and variation of growing rate and lag time with pH for *Lb plantarum*

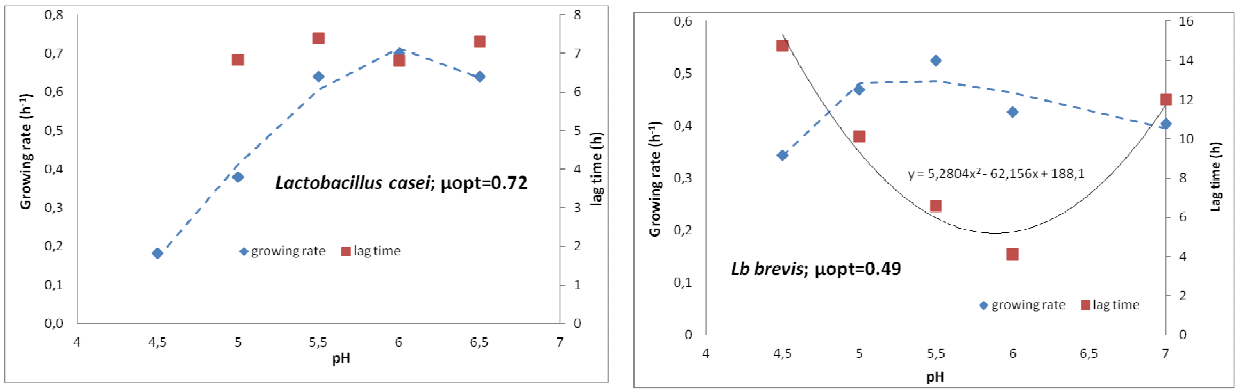


Figure 7. Variation of growing rate and lag time with pH for *Lb casei* and *Lb brevis*

Lb plantarum presents the higher growing stability with pH variation; it can grow under pH 4. *Lb brevis* was the most sensitive to pH, with in particular a lag time that dramatically increases for medium pH.

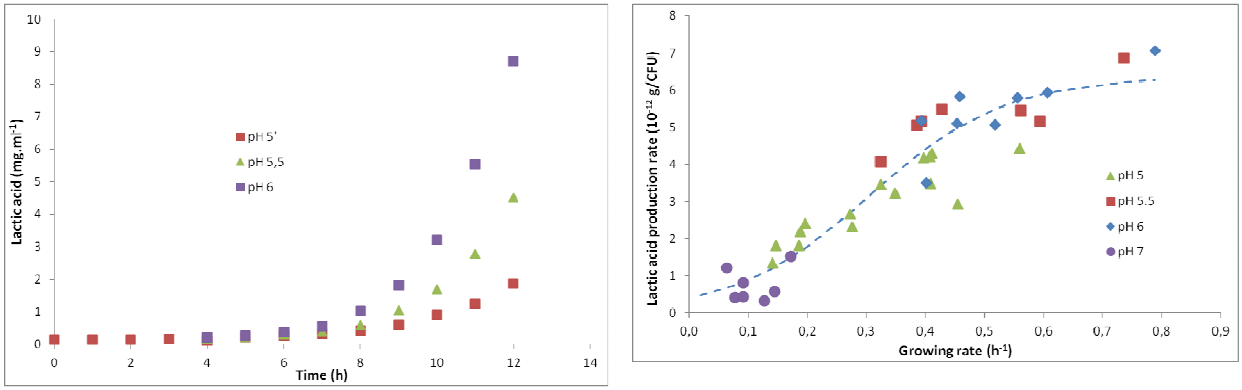


Figure 8. Lactic acid production by *Lb plantarum*

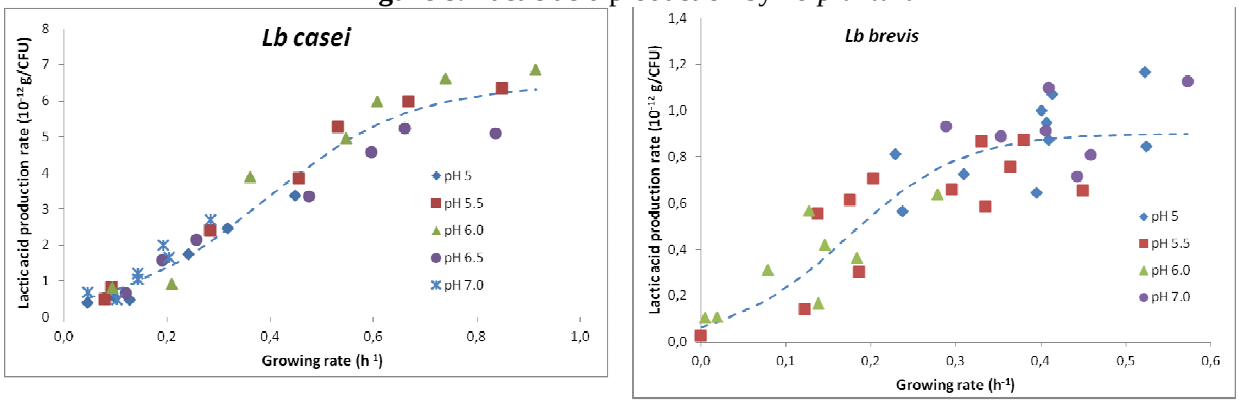


Figure 9. Lactic acid production by *Lb casei* and *Lb brevis*

In MRS medium, the 3 strains exhibited a homolactic fermentation behavior. Lactic acid production was followed for each strain and pH condition (Figure 8). Lactic acid production by cell unit increases with growing rate (μ, Figures 8 & 9) whatever the pH of the medium. A sigmoid model (reaction 2) was fitted between lactic acid production and growing rates; maximum lactic acid production rate was of 6.4, 6.5 and 0.9 10^{-12} g/CFU for *Lb plantarum*, *Lb casei* and *Lb brevis* strains, respectively. *Lb plantarum* and *Lb casei* thus presented the same kinetic of acidification for each living cell whereas *Lb brevis* strain produces seven time lower lactic acid.

A titration curve of malt with lactic acid (reaction 3) was adjusted with an exponential model (Figure 10). The effect of pH on amylase activity was assayed by using a colorimetric substrate (Figure 11). Amylase activity dramatically decreases with pH and was completely null at pH 4. A double linear model (reaction 5) was adjusted to the variation of relative amylase activity with pH.

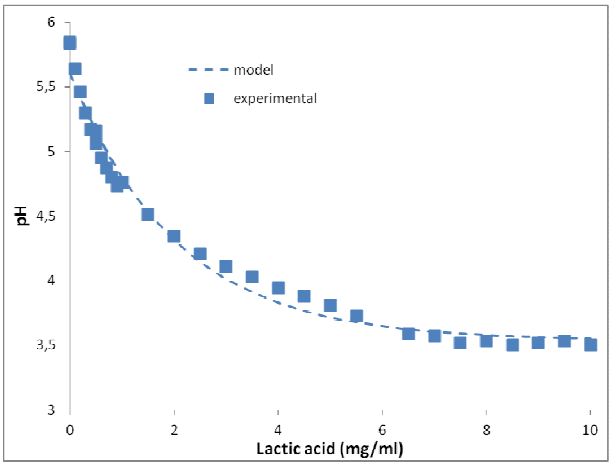


Figure 10. Titration curve of malt

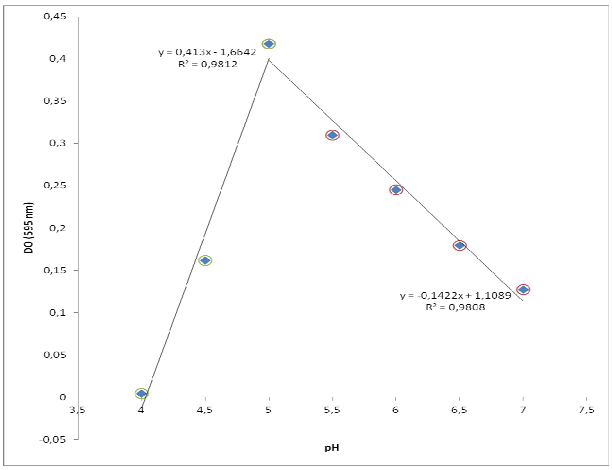
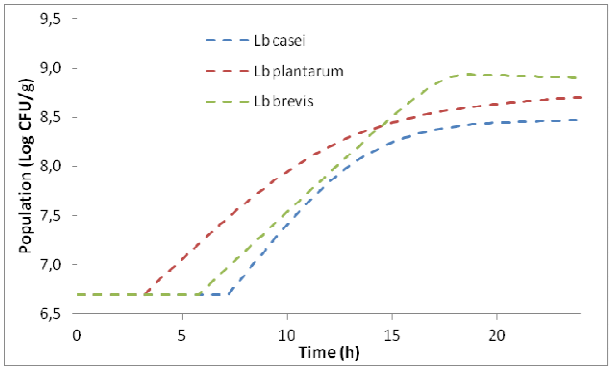


Figure 11. Variation of relative alpha-amylase activity (DO 595 nm) with pH

We also tried to determine *γ(malt)* by inoculating sorghum malt with *Lb* strains. For the moment, this failed as indigenous microbial population of the malt take the advantage on the inoculum. This appears linked to the lag time (over 5 hours) of the inoculum that gives the opportunity to the indigenous population to develop. This problem appears on malt produced at laboratory level, with low bacterial load; it will thus be crucial in the case of traditional malts with *a priori* higher bacterial load. Technological alternatives (improving malt safety, reactivation of strains before inoculation etc) to solve this problem will thus be studied during the project.

When supposing a *γ(malt)* of 1, fermentation can be simulated by the model for a malt inoculated by the three strains at the same level (5 10^6 CFU/g) without pH control.



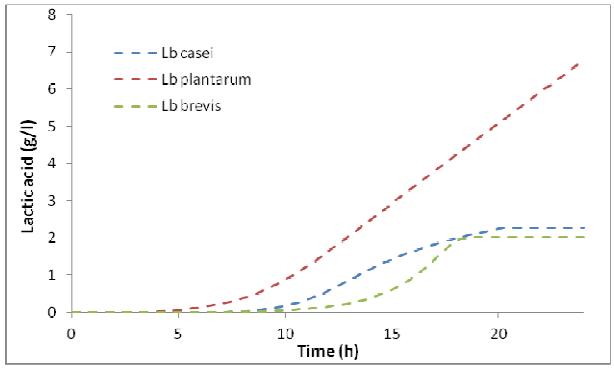


Figure 12. Predicted growing and lactic acid production on malt without pH control

Within 24h fermentation *Lb brevis* will give the highest final population (Figure 12). Lactic acid production will however be much higher for *Lb plantarum*.

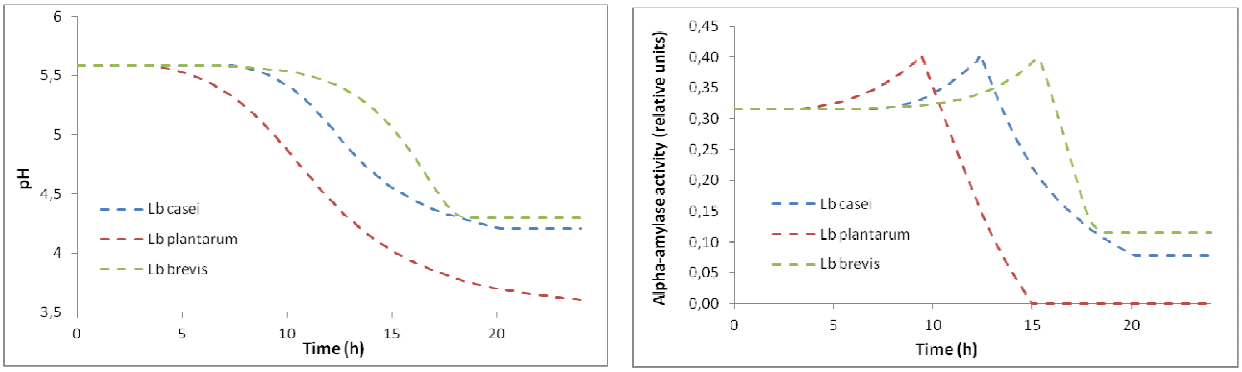


Figure 13. Predicted pH and alpha-amylase activity during malt fermentation

The pH will be safe (under 4.5) within ~10 h when *Lb plantarum* is used for inoculation, but 15-18 h with the other strains (Figure 13). A peak of alpha-amylase activity will be observed when the pH will be of 5 (between 9 and 16 h), and then the activity will decrease to 0 for *Lb plantarum*. A residual activity will remain when the other strains will be used.

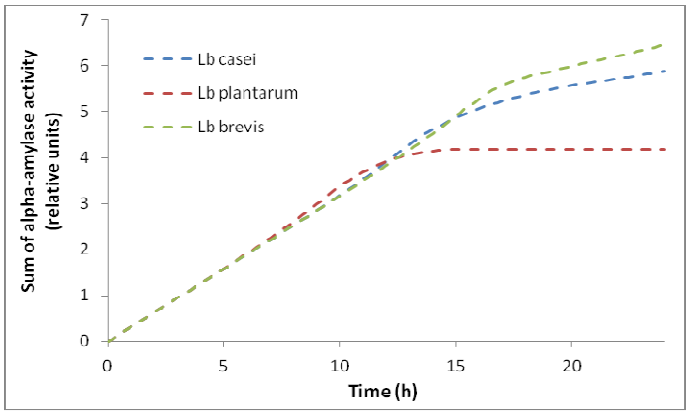


Figure 14. Predicted sum of alpha-amylase activity along malt fermentation

The sum of alpha-amylase activity along the fermentation will represent the total activity to which the sample will be subjected to. The higher it is, the higher the predicted glucose and maltose content. The figure 14 shows that the sum of alpha-amylase activity will be ~ 50% higher when the malt is inoculated by *Lb casei* or *Lb brevis*. This is linked to the lower acidity level at the end of fermentation with these strains.

In conclusion, these first results show that inoculation with *Lb casei* could be the most interesting solution for malt fermentation; it allows rapid acidification for safety, but to a final moderate level that maintains alpha-amylase activity for longer time. The acidification is even slower for *Lb brevis* which appears however too sensitive to the initial pH (very high lag time for neutral pH). *Lb plantarum* gives a too high final acidity.

3 Plan of work

3.1 Optimization of malting

The main objective is to determine the optimal conditions for malting, essentially the duration of unit operations (soaking and germination, table 8). Drying will be set up at 45°C during 12h to limit mould development and amylase degradation. To minimize the growth of moulds, the grains will be washed in a 5g/l sodium chloride (NaCl) solution. Indeed, Gernah et *al.* (2011) reported that the sodium chloride inhibit the growth of moulds. Residual salt within grains will be essayed. Based on the observations during Gowe production (Mestres et al., 2012), the soaking will be performed at 30°C for 8 to 24h. Regarding the germination, the grains will be placed at 30°C for 24 to 72h.

Table 8: Optimization of germination step

Test	Soaking duration (h)	Germination duration (h)
1	24	48
2	8	48
3	20	69
4	12	27
5	20	27
6	12	69
7 ©	16	48
8 ©	16	48
9 ©	16	48

Parameters to be measured: Water content, enzymatic activities, cyanides and tannins content, and mycotoxins.

3.2 Optimization through dehulling of non malted grains and rate of malted flour

Preliminary results showed that a large range of malted grain proportion (20 to 39%) was used for Gowe production. This can be source of variability in the quality of final product. The percentage of malt incorporated should be optimized.

Furthermore, according to Houndelo (2004), the nutritional quality can be also improved by the dehulling of non malted grains. In our study the non malted grains will be dehulled. The malt obtained in the optimum condition in the previous experiments (3.1) will be used. Then, the combination of the ratios of malted/non malted and dehulled/non dehulled grains will be tested as indicate in the experimental design (Table 9). Each flour obtained will be used to produce Gowe according to Vieira-Dalodé et *al.* (2008).

Parameters to be measured are pH, dextrins, starch content, enzymatic activities, sugar and organic acids, viscosity, colour on the flour and acceptability for the Gowé.

Table 9: Experimental design for malted grain and dehulled grain proportions

Test	Malted grains (%)	Dehulled/non malted grains (%)
1	10	90
2	50	50
3	25	75
4©	30	70
5©	30	70
6©	30	70

3.3 Optimization of the saccharification step

Enzymatic activities (amylases) are temperature dependent. Indeed, Biazus et *al.* (2009) reported that the optimum temperature for the α-amylase is 50°C. Nivedita et *al.* (2011) showed that the optimum temperature for amylase activities was 75°C. Vieira-Dalodé et *al.* (2008) suggested the mashing of the sorghum malt slurry at 40°C for 2h for enhancing fermentable sugars. The results of this saccharification were similar to the traditional first fermentation of 12h at 28-30°C (control). In our study, the temperature will vary from 40 to 70°C and the saccharification duration between 1 to 3h (table 10). The couple 40°C / 2h will be used as the control.

Parameters to be measured: pH, starch content, dextrin, enzymatic activities, sugar and organic acid contents.

Table 10: Experimental design for saccharification

Test	Temperature (°C)	Duration (h)
1	70	1
2	70	2
3	70	3
4	60	1
5	60	2
6	60	3
7	50	1
8	50	2
9	50	3
10	45	1
11	45	2
12	45	3
13 (control)	40	2

3.4 Effect of pre-cooking

The processing of Gowe includes a pre-cooking step of a portion of non malted flour (mid cooking of the slurry). The obtained paste is mixed with non-cooked non malted flour and saccharified malted slurry to make dough ready for fermentation. The best proportion of saccharified malted slurry experienced in 3.2 will be used in this experiment. The ratio of cooked non malted flour/non-cooked non malted flour is traditionally of 1/4 (Vieira-Dalode et *al.,* 2007). The main objective of this study is to determine the ratio of cooked flour used in fermentation. Several ratios of cooked flour

/ non-cooked non malted flour will be tested (table 11). Consequently Gowe will be produced from these different ratios.
Parameters to be measured: Starch content, dextrin, enzymatic activities, sugar and organic acids.

Table 11: Experimental design for cooking

Test	Cooked non malted flour (%)	Non cooked non malted flour (%)
1	20	80
2	(30)	(70)
3 ©	(40)	(60)
4 ©	50	50
5 ©	50	50

3.5 Fermentation

Preliminary works (see paragraph 2), showed that *Lactobacillus plantarum* was not a good candidate for Gowé fermentation since it rapidly increases acidification which induces inhibition of residual alpha amylase. They established that inoculation with *Lb casei* could be the most interesting solution for malt fermentation

Vieira-Dalode et *al.* (2007) reported that the dominant microorganisms of Gowe fermentation were the lactic acid bacteria (*Lactobacillus fermentum* and*Weissella confusa, Lactobacillus mucosae and Pediococcus acidilactici*) and yeasts (*Kluyveromyces marxianus, Pichia anomala).* Vieira-Dalode et *al.* (2008) used these different species alone or in combination as starter culture for Gowe fermentation. According to them, Gowe obtained within 7h using *Lactobacillus fermentum* inoculums was similar to the product obtained by spontaneous fermentation. Furthermore, there is a need to test these starters cultures identified in Gowe fermentation alone or in combination (Lactic bacteria/ lactic bacteria and lactic bacteria / yeasts) to complete validate previously model.

Based on those works, two species *Lb casei* and *Lb fermentum* will be tested as inocula at first. These strains will be bought in the commercial forms with the objective of validating models established during fermentation of sorghum to predict the occurrence of lactic fermentation on the final quality of Gowe.

Thereafter, yeasts (*Kluyveromyces marxianus, Pichia anomala)* will be used in combination with both bacteria The highest lag time of the inoculum in malt will be solved by reactivation of strains in sterilized malt before inoculation in malt. Parameters to be measured are pH, microbial count, lactic acid, enzymatic activities.

The best starter from previous test will be kept as inoculum enrichment for the production of Gowe

3.6 Presentation forms of Gowe

Another constraint is related to the shelf-life of the product. The cooked gowé has a water content varying from 71.3% to 82.2%. Combined with the fact that the cooked gowé is packaged in leaves, sometimes under non-hygienic conditions, this high moisture content does not ensure a long shelf-life and the safety of the product. Consequently a study is needed to

improve the shelf life of the product for the national market and to give the opportunity for international market.

Two forms will be presented for commercialization: ready-to-cook flour and ready to consume liquid product. The first form will be destined to the national and international market whereas the second form will be sold on the national market.

Regarding the ready-to-cook flour, the sourdough will be dried at 70°C within 6h as observed by Houndélo (2004) and Bako et *al.*, (2011) who reported that the best characteristics of dried Gowe were obtained from these technological parameters of drying.

Regarding the wet/ yoghurt form, the rate of sugar, milk, water and sourdough necessary to obtain an acceptable yoghurt by the consumers will be determined through the hedonic test of the different Gowe obtained from different formulation. The physico-chemical and microbiological qualities will also be determined in order to insure that it is healthy before tasting. Effect of shelf-life on physico-chemical, microbiological and sensory characteristics will be monitored.

A third form, ie vacuum packaging of Gowe, can also be tested as an alternative between traditional and yoghurt forms. In this case, cooked Gowé will be sold as it is and consumers will prepare the beverage (by adding water and sugar) by their own, like for the traditional product.

For each new form, a name and a price will be proposed and a market test will be realized.

Annex 3 – detailed report for Kenkey

1 Diagnostic on opportunities and re-engineering option

1.1. Market opportunities and consumer demand

The D.1.1.2.1 survey revealed that three main types of kenkey are encountered: Ga and Fanti kenkey, whole maize kenkey (that differ about salt addition, fermentation and cooking duration), and dehulled keneys (Akporhie/Nsiho) or "white kenkey".

Consumer tests in Ghana showed that consumers look for a white, non sticky kenkey with less sour taste and fruity aroma. In addition, consumers often complain about kenkey ball size (too large). Consumer tests performed in EU showed that kenkey will be rejected by non Ghanaian and European consumers.

In consequence, 4 types of products will be developed: improved traditional Ga kenkey and bottled mash Ga kenkey for national market, improved white kenkey and bottled mash white kenkey for international market (Table 1).

Table 1. Kenkey market opportunities

Target Consumer		Type of product	Main characteristics	Type of enterprise	Code
National level	Low income level	Improved Ga kenkey	Improved shelf life (3 months) and safety Low price	Small scale traditional units[1]	Nat1
	High income level	Improved bottled Ga mash		Medium[2] and large[3] scale traditional units Yogurt processors	Nat2
International level	Diaspora	Improved White kenkey with extended shelf life	Garantied safetiness and shelf life (3 monthss at ambiant temperature)	Large scale traditional units New semi-industrial	Int1
	Europeans	Improved bottled mash white kenkey		Yogurt processors	Int2

1.2. Processing parameters and processing constraints

The main problems encountered during processing are safety (during steeping, grinding, fermentation, moulding), reproducibility (fermentation, cooking of aflata), duration (steeping and cooking) and product shelf life (Table 2).

Table 2. Kenkey processing constraints

Code	Raw material	Steeping	Milling	Fermentation	Pre-cooking (Aflata)	Moulding/ packaging	Cooking
Nat1	Grain quality (free of aflatoxins, dusts etc) Safetiness	Duration Safety	Price/duration? Reproducibility	Duration Reproducibility Safety	Labor intensity Level of pre-cooking Reproducibility	"Primitive" packaging	Duration of cooking, reproductibility Shelf life of cooked product
Nat2						Settling of particles.	Shelf life of bottled product.
Int1						Adapted packaging for export	Duration of cooking, reproductibility Shelf life of cooked product
Int2						Settling of particles.	Shelf life of bottled product

1.3. Physicochemical modifications during processing and physicochemical targets

Table 3. Physico-chemical modifications during kenkey processing and targets to achieve after re-engineering

Code	Step							
	Raw material	dehulling	Steeping	Grinding	Fermentation	Pre-cooking (Aflata)	Moulding/ packaging	Cooking
Nat1	Free of aflatoxins Free of foreign material Moisture (10-12%)	Moisture (10-14%)	Moisture (30-35%) Free of mycotoxins Free of pathogens	Particule size (< 250 µm)	pH (3.7-4.2), acidity(~1% lactic acid equivalent, db) sugar levels Free of pathogens	Gelatinization level (XX %) Ratio	Price and affordability of new package	Gelatinization level (XX %) Sugar and lactic acid levels Low stickiness Safetiness Few evolution with storage
Nat2								
Int1		lipid and fibre content (< XX %)					Price and affordability of new package	Gelatinization level (XX %) Sugar and lactic acid levels Safetiness Low stickiness or viscosity Few evolution with storage
Int2								

1.4. Re-engineering options

Re-engineering is focusing on steeping process (to shorten and secure it) and on fermentation and precooking as shown in table 4. The possibility of eliminating pre-cooking will be investigated to see its effect in ensuring quality (i.e. improving reproducibility), decreasing stickiness and labour intensity. Concerning fermentation, it will in particular pay attention to the kinetics of acidification that should be rapid in a first step for insuring safety but slow, in a second step to increase shelf life (consumers look indeed to products with low acidity). In this regard the development and use of starter culture is being investigated and is reported in detail in D.2.1.1. In the case of bottled mash kenkey, addition of amylase to prevent settling during storage will be tested. New forms and size of kenkey will be developed for national and international markets. Packaging of kenkey in synthetic material rather than the traditional leaves will also be investigated. Finally, cooking will be optimized to shorten it and increase shelf life of the product.

Table 4. Kenkey re-engineering options

Code	Raw material	Steeping	Grinding	Fermentation	Pre-cooking (Aflata)	Moulding/ packaging	Cooking
Nat1	Improve maize sourcing	Optimize steeping duration depending on material partcule size Inoculation with LaB to preserve from pathogens		Inoculation with LaB and yeasts (?) starter cultures or Backsloping Optimize duration, temperature, water content	Optimize (proportion, temperature, duration, water content, mixing intensity), or Remove this operation	Test synthetic material Change shape and dimension (moulds)	Optimize: open or pressure steam cooking
Nat2						Test enzymatic treatment (amylase) to prevent settling/sedimentation	
Int1			Cracking of grains			Test synthetic material Change shape and dimension (moulds)	Optimize: open or pressure steam cooking
Int2						Test enzymatic treatment (amylase) to prevent settling/sedimentation	

2 First results

2.1 Steeping and fermentation

Influence of steeping and fermentation times on moisture content, pH, acidity, organic acid and sugar content, colour and texture of laboratory prepared white Kenkey was studied using

a 3x3 factorial design of steeping (12h, 30h, & 48h), fermentation (0h, 12h & 24h) . Figure 1 shows general trends of variation for some of quality criteria.

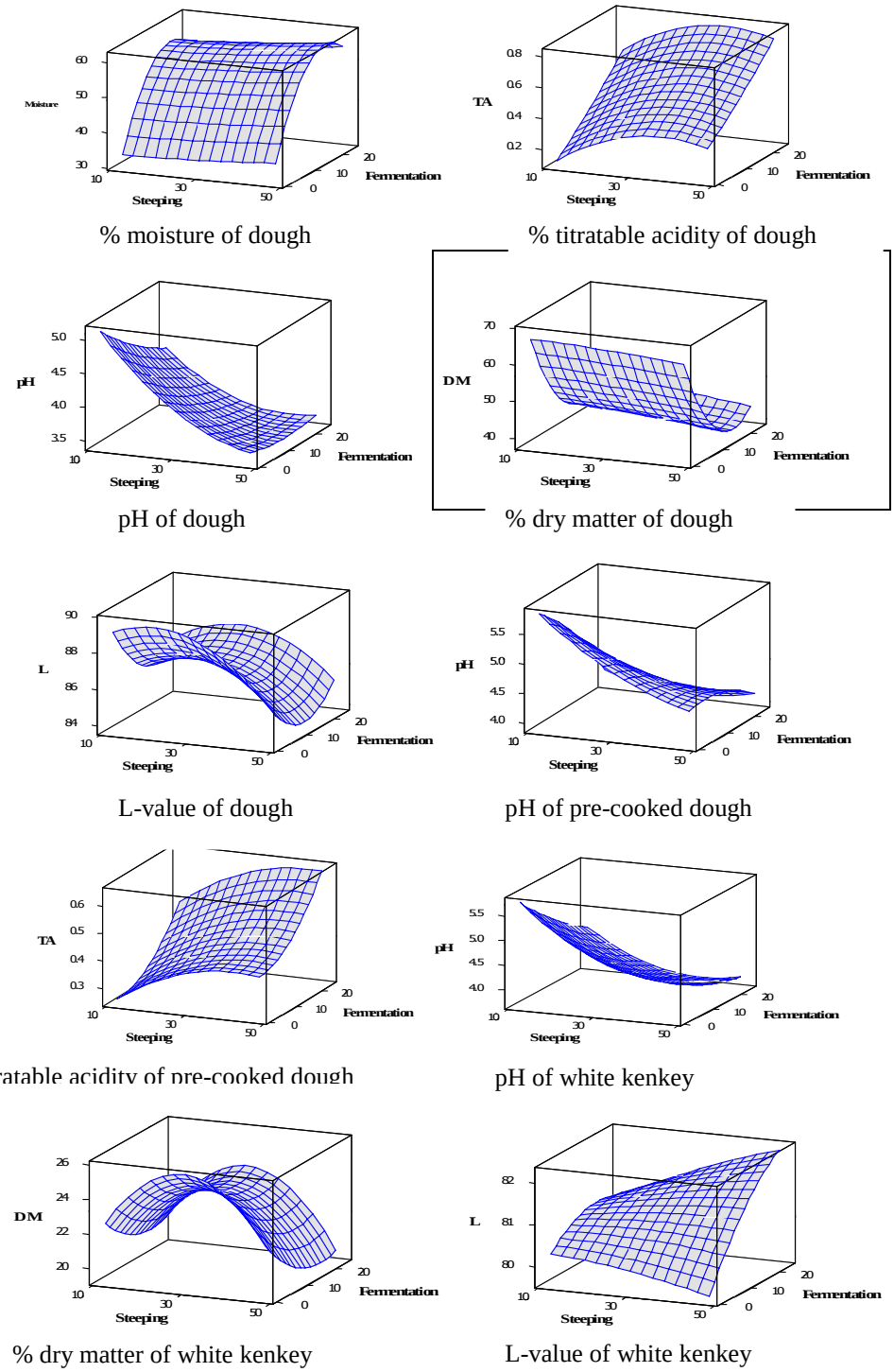


Fig. 1. Surface plots of kenkey and intermediary products against steeping and fermentation duration.

Figure 2 shows that when steeping time of the dehulled maize grains was increased from 12 to 30 h, the moisture content of the grains increased significantly. In the milled steeped grains kneaded with water, the 48 h steeped grains always recorded slightly higher moisture content and the 30 h steeped grains the least during further processing into kenkey.

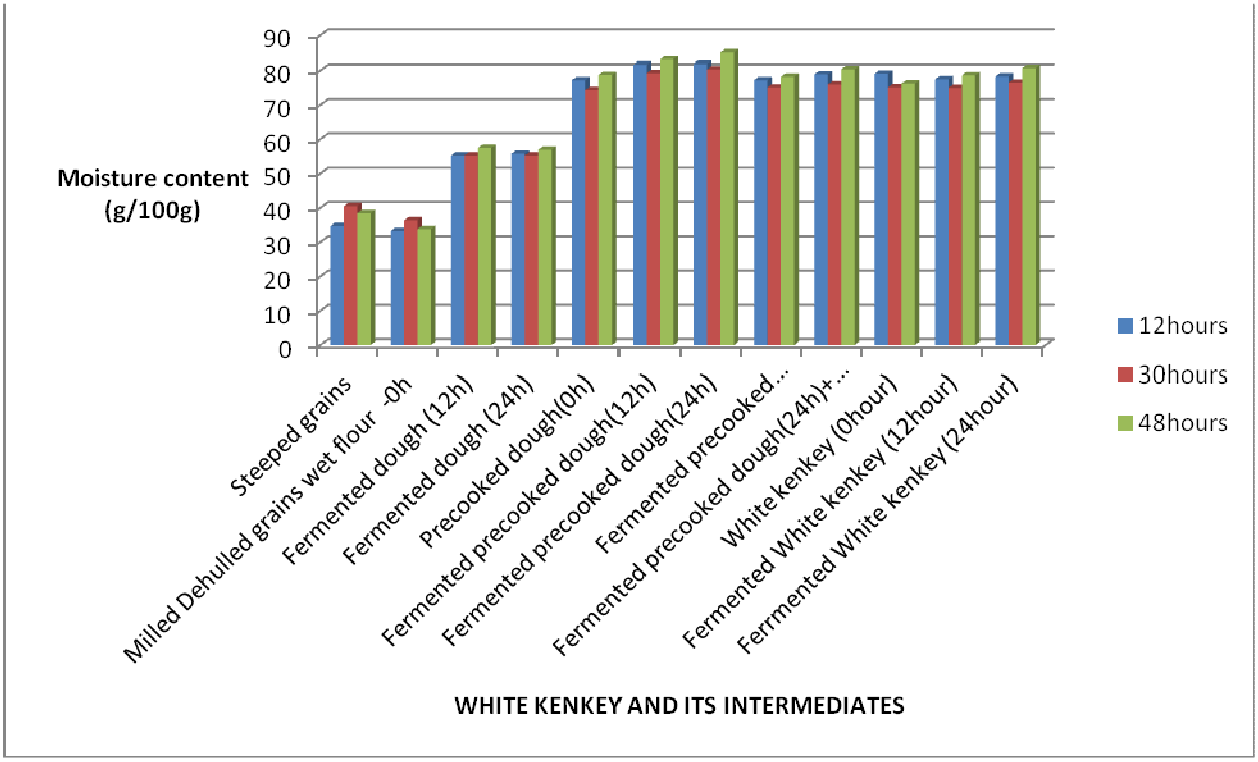


Figure2. Effect of steeping times and dough fermentation on moisture of intermediate products and white kenkey

Dehulled maize grains steeped for 12 recorded a significantly higher pH value than the grains steeped for 30 or 48 h at the 95 % confidence level (figure 3) obviously due to more prolonged activity of the lactic acid bacteria involved in the fermentation. There was however no significant difference in pH between steeping for 30 or 48 h. Similarly, unfermented samples showed higher pH than 12 to 24 fermented products, which were not significantly different.

The increase in pH observed after pre-cooking and cooking may have resulted from loss of volatile acids by evaporation.

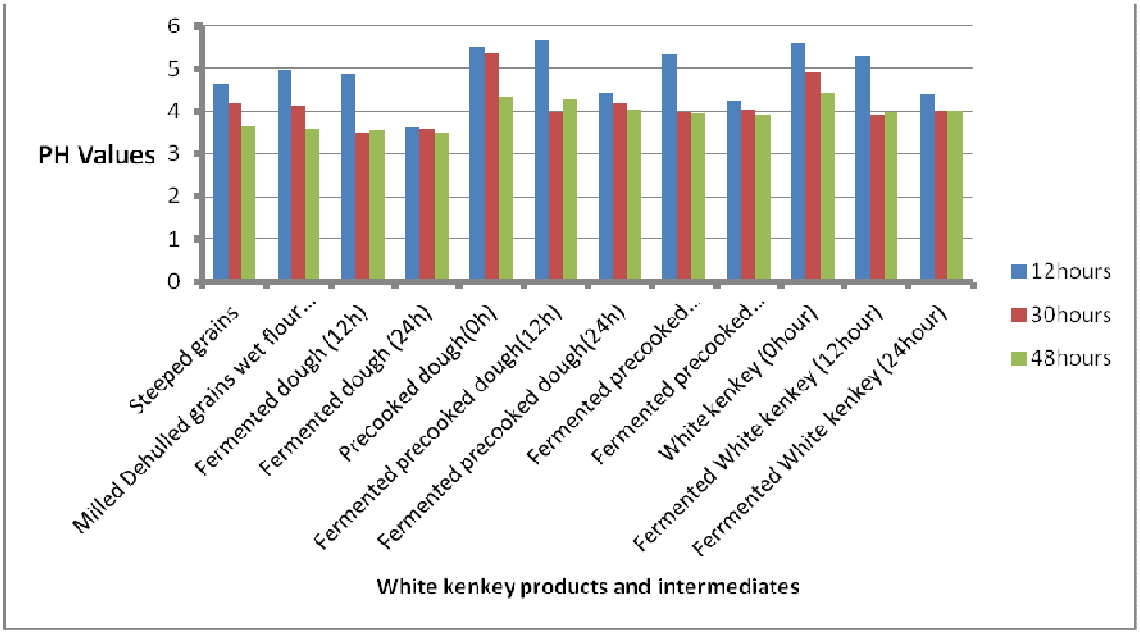


Figure 3. Effect of steeping times and dough fermentation on pH of intermediate products and white kenkey

With regards to titratable acidity, increasing the steeping time also resulted in an increase in titratable acidity (Figure 4). In addition, TA also increased with fermentation time and was higher for 24 h fermentation all along the process.

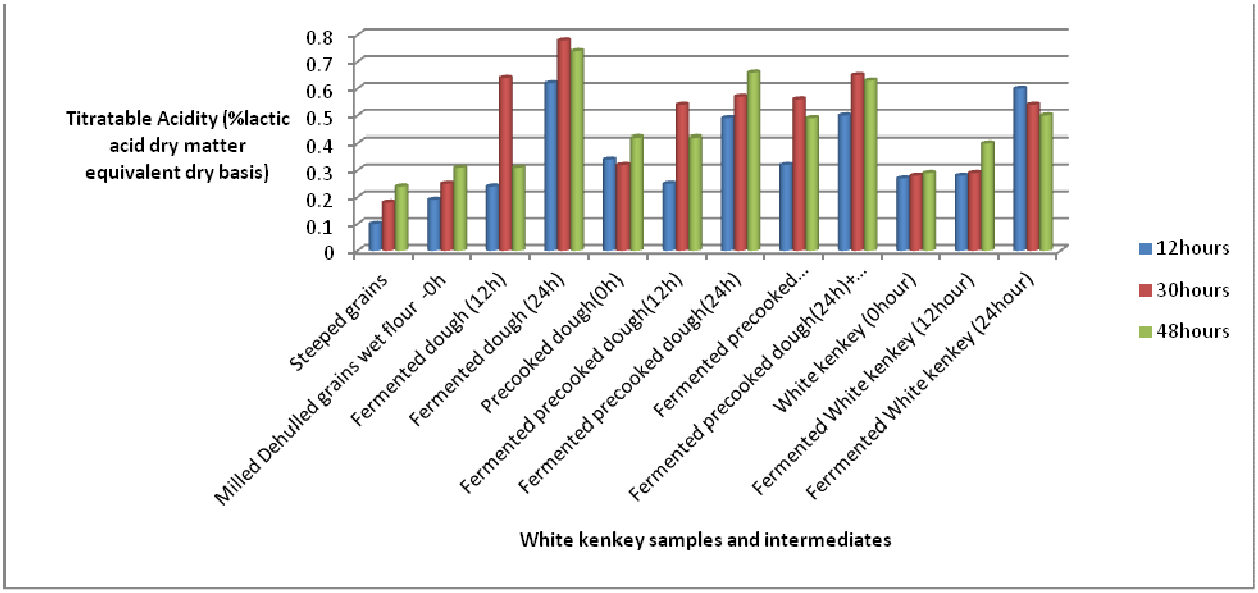


Figure 4. Effect of steeping times and dough fermentation on titratable acidity of intermediate products and white kenkey

Organic acids and sugars will be determined during November and December.

The effect of steeping time and fermentation time on the colour of dehulled maize grains, kenkey and intermediary products are shown in Fig 5 and Tables 5a and 5b. There was no significant difference between the lightness of fermented doughs (Figure 5).

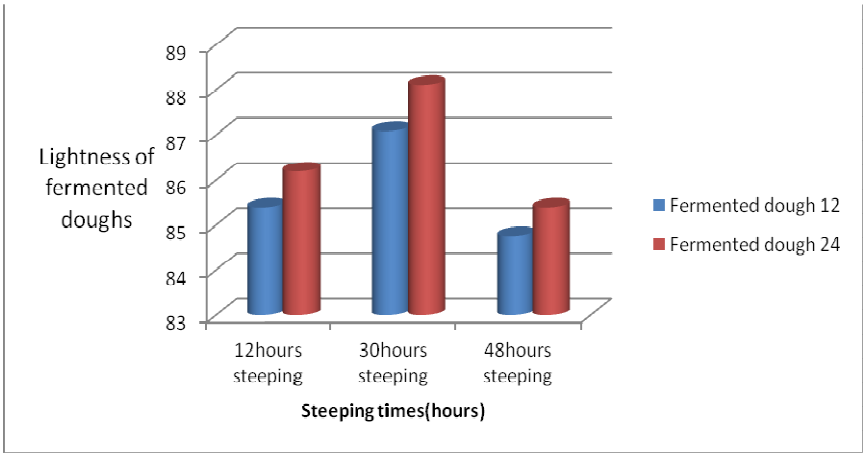


Figure 5. Effect of fermentation on lightness of fermented dehulled maize dough steeped at three durations

Table 5a. Effect of steeping time and dough fermentation on the colour of before and after dough fermentation

Colour		12 h steeping	30 h steeping	48 h steeping
Intermediate moisture meal				
L		88.97 ± 0.86a	88.37 ± 0.54a	86.89 ± 2.29b
ΔE		10.06 ± 0.81	10.56 ± 0.47	11. 89 ± 2.40
Fermented dough				
L	12 h fermentation	85.39 ± 0.16	87.09 ± 0.55	84.76 ± 2.02
ΔE		13.20 ± 0.15	11.56 ± 0.67	12.41 ± 0.22
L	24 h fermentation	86.19 ± 1.19	88.11 ± 0.33	85.39 ± 2.49
ΔE		12.44± 1.18	10.79 ± 0.35	13.27 ± 2.71

Table 5b. Effect of steeping time and dough fermentation on the colour of white kenkey

Colour			12 h steeping	30 h steeping	48 h steeping
White kenkey	0 h fermentation	L	80.29 ± 0.86	80.24 ± 0.36	77.60 ± 0.76
		ΔE	18.23 ± 0.94	18.14± 0.36	20. 68 ± 0.75
	12 h fermentation	L	80.63 ± 0.26	81.43 ± 0.28	81.42 ± 0.07
		ΔE	17.93 ± 0.13	17.04± 0.24	17.13 ± 0.01
	24 h fermentation	L	82.68 ± 0.22	81.59 ± 0.51	81.99 ± 0.62
		ΔE	15.88 ± 0.32	16.99 ± 0.51	16.64 ± 0.61

Texture was measured using TA-XT2 Texture Analyzer using a penotrometer with 6mm diameter; stickiness was the energy necessary for removing the penetromer from kenkey after compression. Stickiness clearly decreased as steeping duration increased from 12hour, 30hours to 48hours (Figure 6).

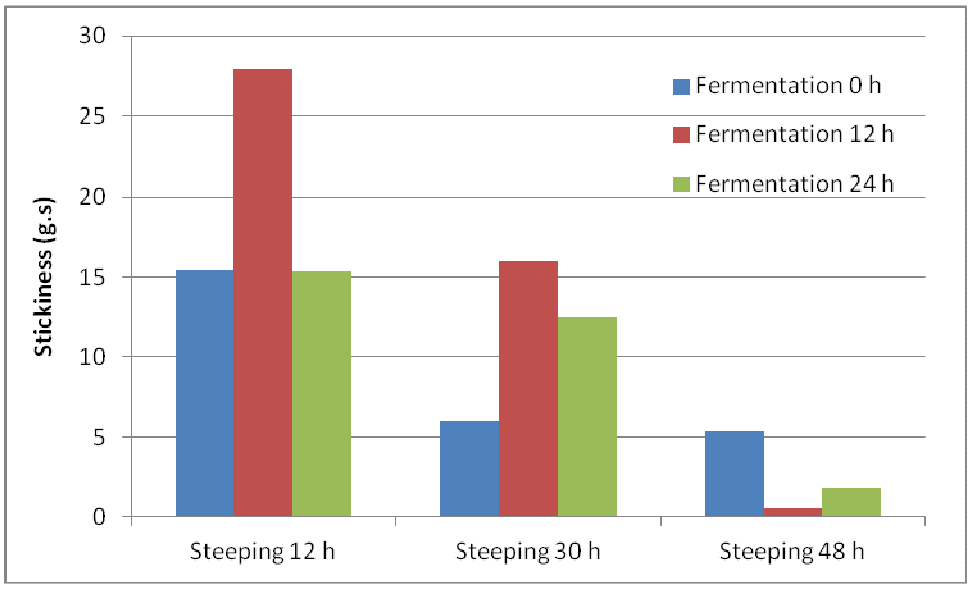


Figure 6. Stickiness of Kenkey measured with texture analyzer

The pasting characteristics of fermented dough used to prepare white kenkey are presented in Table 6.

Table 6. The pasting characteristics of dough prepared from dehulled maize steeped for different periods and dough fermented for different durations

Sample	Pasting temp (°C)	Peak viscosity (BU)	Viscosity at 95 °C (BU)	Viscosity after 15 min at 95 °C (BU)	Viscosity at 50 °C	Pasting stability at 50 °C (BU)	Setback
Dough from 48 h steeped grains	54.10	311.5	264	151	368.5	340.5	217
12 h fermented dough from 12 h steeped grains	79.7	189.5	178.5	159	362.5	347.5	203
12 h fermented dough from 30 h steeped grains	74.03	359	311	187	418	430	230
24 h fermented dough from 48 h steeped	75.2	351.50	289	196.5	499 .80	470.5	301.50

2.2 Optimizing steeping temperature and steeping and fermentation times

The effect of steeping temperature, steeping time, and fermentation time on critical dough variables and kenkey attributes was investigated using Response Surface Regression procedures (SAS, Version 9.2). To optimize the range of experimentation the Box Behnken design was used (Table 7). The critical processing variables used were; Steeping time (12, 30, 48 h), steeping temperature (25, 35, 45 °C) and fermentation time (0, 12, 24 h). For each experimental batch a known weight of dehulled maize was steeped in water at room temperature and water bath, milled and fermented for various durations

Table 7: Box Behnken design for steeping time, steeping temperature and fermentation time

Run	Steeping times (hour)	Steeping temperature (ºC)	Fermentation time (hours)
1	12	45	12
2	12	35	0
3	48	35	0
4	48	35	24
5	12	35	24
6	30	45	0
7	30	35	12
8	30	25	0
9	30	35	12
10	30	35	12
11	12	25	12
12	30	45	24
13	48	45	12
14	30	25	24
15	48	25	12

This experiment is on-going and data will be compiled and computed after.

2.3 Use of starter cultures

White kenkey were prepared using inoculation with *Lb. fermentum*, *Lb. brevis*, *C. krusei*, and *S. cerevisiae*, alone or in combined (see D 2.1.1). The level of inoculation was 10^7CFU/g for LAB and/or 10^6 CFU/g for yeasts and the fermentation time was of 24 h.

In inoculated fermentations, the use of any of the cultures tested resulted in a more rapid drop in pH due to a faster production of acid than in the uninoculated control sample. The use of LAB resulted in a more rapid drop in pH than use of the yeasts. The isolates which showed the highest rate of acidification at various stages of steeping and dough fermentation were *Lb. fermentum* 2 and *Pediococcus pentosaceus*. White kenkey produced using various starter cultures were all acceptable to an untrained 20 member taste panel who scored between 6.0 and 7.0 on a nine point hedonic scale for all products. The highest score for acceptability of 6.73 which represented like moderately was scored for white kenkey prepared with a mixed culture comprising *Lb. fermentum*, *Lb. brevis*, and *C. krusei*. (Table 8). PCA bi-plot showed that kenkey fermented with starter cultures which included *Lb. brevis* tended to have a good taste and enhanced their acceptability by the taste panel.

Table 8. Sensory evaluation of kenkey made using fermented dough by selected culture

Starter culture	Odour	Taste	Texture	Overall acceptability
Control	6.23 ± 0.18	6.40 ± 0.00	6.33 ± 0.11	6.30 ± 0.07
L. brevis	6.63 ± 0.04	6.65 ± 0.42	6.83 ± 0.32	6.58 ± 0.67
L. fermentum	6.65 ± 0.21	6.28 ± 0.60	6.25 ± 0.42	6.25 ± 0.35
C. krusei	6.80 ± 0.35	6.53 ± 0.04	6.73 ± 0.30	6.33 ± 0.46
L fermentum + S. cerevisae (FC)	6.38 ± 0.18	6.50 ± 0.28	6.78 ± 0.04	6.33 ± 0.46
L. fermentum + C. krusei (FK)	6.38 ± 0.18	6.53 ± 0.11	6.63 ± 0.11	6.23 ± 0.53
L. brevis + C. krusei (BK)	6.73 ± 0.25	6.43 ± 0.11	6.75 ± 0.14	6.30 ± 0.14
L. ferm + S. cere + C. krusei (FCK)	6.58 ± 0.39	6.28 ± 0.18	6.40 ± 0.07	6.00 ± 0.00
L. ferm + L. brev + C. krusei (FBK)	6.00 ± 0.07	6.65 ± 0.57	7.08 ± 0.46	6.73 ± 0.67
L. ferm + L. brev + C kru + S. cere (FBKC)	6.18 ± 0.18	6.35 ± 0.49	7.09 ± 0.25	6.28 ± 0.18

2.4 Moulding and packaging

Preliminary studies conducted into remoulding (reshaping) and packaging of kenkey proved successful for remoulding but not yet for packaging. Packaging kenkey in sausage casing instead of leaves proved unsuccessful as the kenkey could not retain its shape and the casing also got punctured during steaming. Remoulding/reshaping kenkey proved very successfully as cylindrically shaped kenkey were much more pleasing to the eye than the traditional round balls (Figure 7)

Traditional forms of Kenkey

New shapes of kenkey (re-engineering)

Figure 7. Shape of traditional and re-engineered Kenkey

3 Plan of work

	Programmed Activities	2012			2013											
		O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
Steeping	1. Optimize steeping duration depending on material particle size															
	2. Inoculation of steep water with LaB and yeasts to prevent pathogens and for fruity aroma															
Milling	3. Dry milling before steeping/ cracking of grains before steeping															
Fermentation	4. Test second inoculation with LaB and yeasts starter cultures or Backsloping															
	5. Optimize duration, temperature, water content															
Precooking (Aflata)	6. Optimize (proportion, temperature, duration, water content, and mixing intensity).															
	7. Test elimination of aflata for some products.															
Moulding and packaging	8. Test synthetic material , change shape and dimension															
	9. Test synthetic material															
	10. Change shape and dimension (moulds)															
	11. Test enzymatic treatment (amylase) to prevent settling/sedimentation															
Cooking	12. 13.Optimize: open or pressure steam cooking															

Annex 4 – detailed report for KS

1 Diagnostic on opportunities and re-engineering option

1.1. Market opportunities and consumer demand

Concerning KS, the diagnostic is not complete due to Egyptian Revolution that impeded the technological survey of the main process types. Nevertheless, based on WP5 deliverables, two types of products will be developed (Table 1):

- improved traditional KS (including new shape, size and presentation of KS) for national markets and the tourist industry
- a variety of products derived from final and intermediate processes in Kish Sa'eedi KS production. The advantages of KS such as the affordable price, the relatively high nutritional quality, the low energy input required for its production and the low environmental impact of its production method, could lead to commercial exploitation as follows:
    - Ready-to-eat food items e.g. the snacks that are popular among both the food processing companies and the consumers offer excellent opportunities for diversification by production of a school snack, convenience food, energy bar, breakfast cereal, crunchy snack and savory crisp.
    - A variety of intermediate KS products offered to consumer's e.g. fresh or frozen KS sourdough packed in modified atmosphere packs and used as a thickening ingredient in stews and gravies.
    - Instant or ready to use dehydrated KS sourdough to be used in the production of baby foods.

Based on the KS sensory evaluation, KS with off-white to light creamy color, moderate sourness, pleasant typical KS aroma and crunchy texture is more appreciated by traditional consumers.

Table 1. KS market opportunities

Target Consumer		Type of product	Main characteristics	Type of enterprise	Code
National level	Low and high income level Expatriate and tourist communities	Traditional KS balls/nuggets	Consistent organolpetic properties and improved safety product Low price High nutritional value Long shelf life	Small scale traditional units[1]	KS1
International level	Diaspora and Europeans	Energy bar Crunchy snack Breakfast cereal KS sourdough	Improved diversity and way of consumption Improved organoleptic properties Guarantied safetiness	Large scale traditional units[2] New semi-industrial units	KS2

[1] < 20 kg/month, [2] 50-100 kg/month

1.2. Processing parameters and processing constraints

Main processing constraints are listed in Table 2. They will be updated by the completion of technological survey and analysis of product during processing.

Table 2. KS processing constraints

Code	Step							
	Wheat parboiling	**Drying**	**Grinding**	**Laban Zeer**	**Mixing/ kneading**	**Fermentation**	**2nd kneading Shaping**	**Drying**
KS1	Over or under cooking	dust or insect infestation	Difficulty to grind?	Worm infestation Unpleasant odor Discoloration Safe from contaminants	Texture is doughy and sticky	Unpleasant odor Safetiness	Texture is too doughy and sticky	Worm infestation Safetiness
KS2								

1.3. Physicochemical modifications during processing and physicochemical targets

Some data about KS physico-chemical modifications during processing are indicated in Table 3. Completion of the measurement will follow through the new KS production season of 2013

Table 3. Physico-chemical modifications during KS processing and targets to achieve after re-engineering

Code	Step							
	Wheat parboiling	**Drying**	**Grinding**	**Laban Zeer**	**1st Mixing/ kneading**	**Fermentation**	**2nd kneading Shaping**	**Drying**
KS1	Gelatinization level (40-70 %)	final moisture percentage	Particule size (17-20% < 75µm)	pH, acidity Free of pathogens	water content	pH, acidity Free of pathogens	typial doughy texture	water content Free of pathogens
KS2								

1.4. Re-engineering options

Re-engineering options are listed in Table 4. They will be updated by the completion of technological survey and analysis of product during processing.

Table 4. KS re-engineering options

Code	Step							
	Wheat parboiling	**Drying**	**Grinding**	**Laban Zeer**	**Mixing/ kneading**	**Fermentation**	**2nd kneading Shaping**	**Drying and packaging**
KS1	Optimizing (temperature, duration, water ratio)	temprature and duration of drying	Type of mill, number of passes	Inoculation with LaB or Backsloping Salt adjustment?	Wheat/LZ ratio Water level Mechanization	Inoculation with LaB or Backsloping	water content and duration of hand kneading Mechanization	Ball/nuggets size, duration of drying New packaging
KS2								

It must be emphasized that process changes should take into account the role of the poor who originated and preserved the processes and how they will benefit from the modifications.

Based on the field survey results the standard of hygiene adopted by the KS operatives, the potable quality of the water and the cleanliness of the utensils used are the possible sources of contamination. Upgrading of such factors will consequently improve the hygienic standard of KS production that is traditionally mastered by women. Minimal and adequate guidance, as well as monitoring of product safety are required. Illustrated guidelines to avoid possible unhygienic practices, can serve this goal.

Concerning the fermentations, inoculation with pure starter cultures or backslopping will be tested. The main advantage of natural fermentation processes is that they are fitting to the rural situation, since they were in fact mastered and created by it. The consumer safety of several fermented foods is improved by lactic acid fermentation, which creates an environment that is unfavorable to pathogenic Enterobacteriaceae and Bacillaceae. However, the variety of microorganisms present in a fermented food can create rich and full flavors that are specific to KS are hard to imitate when using pure starter cultures under aseptic conditions.

The equipment needed for the improvement of KS traditional processes can be a challenge in itself. Fermentations carried out in containers (pots, bowls) with unusual surface characteristics such as semi-porous clay, and wood-framed sieves made from interwoven metal-wire mesh which are difficult to replicate. Mechanization of some key processing stages is desirable, for example, the manual mixing of the ingredients (*Laban Zeer*, parboiled ground wheat, salt, and condiments) in traditional KS making can be replaced by using an appropriate mixing machine. Such a replacement will ensure optimal homogeneity of the different ingredients, and avoid possible contamination of the product that may originate from the KS operatives and/or handlers.

Re-engineering will in particular deal with improving shelf life and food safety level of the traditional products. There is scope for improvement of, a) the open air method used for drying to avoid contamination, and b) the packaging of the products after processing. In the traditional methods, The KS is packed (stored) in earthenware jars. In the industrial production of second generation KS derivatives, the packaging and labelling must be done with due care and in compliance to the specific statutory standards. This is important not only for marketing reasons, but also to protect the product from post processing contaminations and/or accelerated deterioration.

Proposing a new shape such as KS flat sheets instead of KS balls or nuggets will also reduce the time required for drying and consequently will reduce possible contamination. In addition, improving the nutritional quality by increasing the respective proportion of the ingredients, namely of *Laben Zeer* to ground parboiled wheat will be experimented. This will increase energy as well as nutrient density and can offer future opportunities for improvement of the attributes of functional KS based foods as KS is high in fiber, low in fat, with a potential for prebiotic action

2 Plan of work

Before a better understanding of the process and the physicochemical characterization of the product (beginning of 2013), the work will start with:

- Trials to produce the typical flavour of fermented milk (*Laban Zeer*) using standardized mixed pasteurized cow and buffalo milk inoculated by selected lactic acid cultures will form the basis for formulation of recommendations on the best incubation temperature and time
- Evaluation of aroma of traditional KS, and of inoculated KS
- Impact assessment of wheat cooking level and wheat/LZ ratio on KS texture, colour and nutritional quality
- Drying tests of KS will be performed in hot air oven with shape and size variations